Original: Japanese
Provisional translation

Analytical Method for Pyrasulfotole (Animal and Fishery Products)

1. Analytes

Pyrasulfotole

(5-hydroxy-3-methyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl] methanone (hereinafter referred to as metabolite M1)

2. Application

Animal and fishery products

3. Instrument

Liquid chromatograph-tandem mass spectrometer (LC-MS/MS)

4. Reagents

Use the reagents listed in Section 3 of the General Rules, except the following.

Reference standard of pyrasulfotole: Contains not less than 98% of pyrasulfotole.

Reference standard of metabolite M1: Contains not less than 98% of metabolite M1.

5. Procedure

1) Extraction

i) Muscle, liver, kidney, milk, egg, fish and shellfish

Add 100 mL of acetone to 10.0 g of sample, homogenize, centrifuge at 2,500 rpm for 5 min and collect the acetone layer. Dissolve the residue in 50 mL of acetone, homogenize, and centrifuge as described above. Collect the acetone layer, combine with the previous acetone layer and add acetone to make exactly 200 mL.

Take exactly a 10 mL aliquot of the solution, add 100 mL of 10 w/v% sodium chloride solution, 5 mL of hydrochloric acid and 50 mL of *n*-hexane, shake for 5 min and remove the hexane layer.

Extract the aqueous layer with shaking twice with 100 mL and 50 mL each of ethyl acetate. Dehydrate the extract with anhydrous sodium sulfate, filter out anhydrous sodium sulfate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetone.

ii) Honey

Dissolve 10.0 g of sample in 20 mL of water. Add 100 mL of acetone to the sample, homogenize, centrifuge at 2,500 rpm for 5 min and collect the acetone layer. Add 50 mL of acetone to the residue, homogenize, and centrifuge as described above. Collect the acetone layer, combine with the previous acetone layer and add acetone to make exactly 200 mL.

Take exactly a 10 mL aliquot of the solution, add 100 mL of 10 w/v% sodium chloride

solution, 5 mL of hydrochloric acid and 50 mL of *n*-hexane, shake for 5 min, and remove the hexane layer.

Extract the aqueous layer with shaking twice with 100 mL and 50 mL each of ethyl acetate. Dehydrate the extract with anhydrous sodium sulfate, filter out anhydrous sodium sulfate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetone.

iii) Fat

Add 100 mL of acetone to 5.00 g of sample, homogenize, centrifuge at 2,500 rpm for 5 min, and collect the acetone layer. Add 50 mL of acetone to the residue, homogenize, and centrifuge as described above. Collect the acetone layer, combine with the previous acetone layer and add acetone to make exactly 200 mL.

Take exactly a 20 mL aliquot of the solution, add 100 mL of 10 w/v% sodium chloride solution, 5 mL of hydrochloric acid and 50 mL of *n*-hexane, shake for 5 min and remove the hexane layer.

Extract the aqueous layer with shaking twice with 100 mL and 50 mL each of ethyl acetate. Dehydrate the extract with anhydrous sodium sulfate, filter out anhydrous sodium sulfate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetone.

2) Clean-up

Inject 10 mL each of methanol and acetone into a graphite carbon/aminopropylsilanized silica gel layered cartridge (500 mg/500 mg) sequentially, and discard each effluent. Transfer the solution obtained in 1) to the cartridge, add 10 mL of acetone and discard the effluent. Then, inject 30 mL of methanol and hydrochloric acid (99:1) mixed solution, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in methanol to make exactly 5 mL and use this solution as the test solution.

6. Calibration curve

Dissolve each reference standard of pyrasulfotole and metabolite M1 in acetone respectively to prepare stock standard solutions. Prepare standard solutions of several concentrations by mixing each stock standard solution appropriately and diluting with methanol. Inject each solution into LC-MS/MS and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of each analyte in the test solution corresponding to 0.01 mg/kg in the sample is 0.001 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS and calculate each concentration of pyrasulfotole and metabolite M1 from the calibration curve prepared in **6**. Use the following equation to calculate the concentration of pyrasulfotole including metabolite M1.

Concentration (ppm) of pyrasulfotole (including metabolite M1) = A + B × 1.040

A: Concentration (ppm) of pyrasulfotole

B: Concentration (ppm) of metabolite M1

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel 2.1 mm inside diameter, 150 mm in length and 5 μm in particle diameter.

Column temperature: 40°C

Mobile phase: Linear gradient from a mixed solution of 5 mmol/L ammonium acetate solution and 5 mmol/L ammonium acetate-methanol solution (17:3) to (1:1) in 14 min, followed by linear gradient to (1:19) in 0.1 min, and hold at (1:19) for 5 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*)

Pyrasulfotole: Precursor ion 363, product ions 251, 144

Metabolite M1: Precursor ion 349, product ions 251, 144

Injection volume: 5 μL

Expected retention time

Pyrasulfotole: 12 min

Metabolite M1: 11 min

10. Limit of quantification

Pyrasulfotole: 0.01 mg/kg

Metabolite M1: 0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extracting pyrasulfotole and metabolite M1 from sample with acetone, washing with *n*-hexane, transferring it into ethyl acetate for re-dissolution, cleaning up with a graphite carbon/aminopropylsilanized silica gel layered cartridge, and quantifying and confirming using LC-MS/MS. In this method, pyrasulfotole and metabolite M1 are quantified respectively. For the concentration of pyrasulfotole including metabolite M1, the concentration of metabolite M1 is converted to the concentration of pyrasulfotole by multiplying by the conversion factor and the sum of the concentration of pyrasulfotole and metabolite M1 is regarded as the analytical result of pyrasulfotole.

2) Notes

i) As pyrasulfotol and metabolite M1 adhere to diatomaceous earth, suction filtration using diatomaceous earth was avoided after extraction, and in addition, some samples may become clogged without diatomaceous earth; therefore, the method was to take the supernatant after centrifugation.

ii) When the analytical method using LC-MS/MS was developed, the following monitoring ions were used:

Pyrasulfotole

for quantitative ions (*m/z*): precursor ion 363, product ion 251

for qualitative ions (*m/z*): precursor ion 363, product ion 144

Metabolite M1

for quantitative ions (*m/z*): precursor ion 349, product ion 251

for qualitative ions (*m/z*): precursor ion 349, product ion 144

iii) Measurement conditions in ESI (−) mode

When the analytical method using LC-MS/MS in ESI (−) mode was developed, the following monitoring ions were used:

Major monitoring ions (*m/z*)

Pyrasulfotole: precursor ion 361, product ion 79

Metabolite M1: precursor ion 347, product ions 267, 64

iv) Food items used to develop the analytical method: cattle muscle, chicken muscle, cattle fat, cattle liver, salmon, eel, *Corbicula* (freshwater clam), cattle milk, chicken egg, and honey.

12. Reference

None

13. Type

C